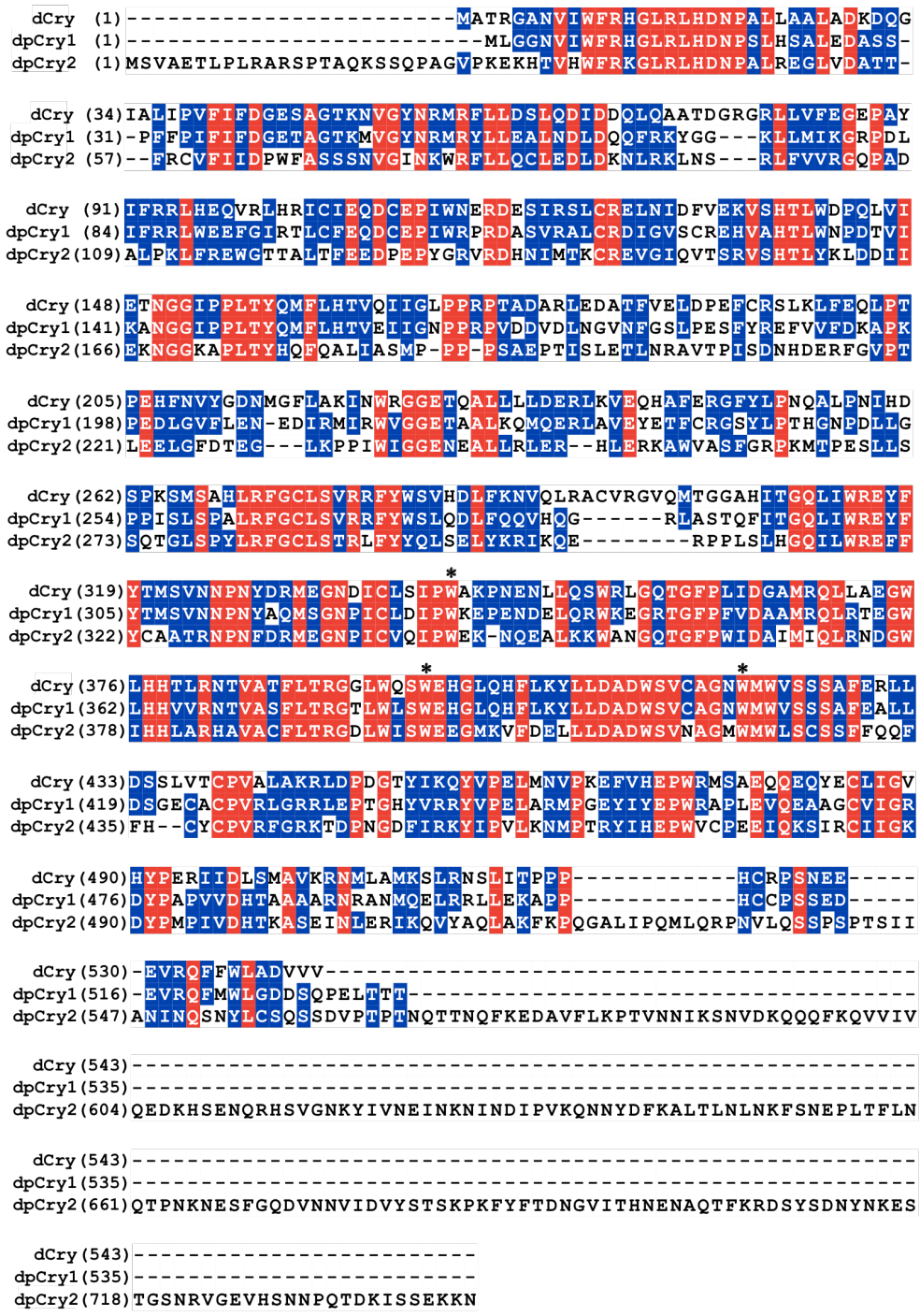
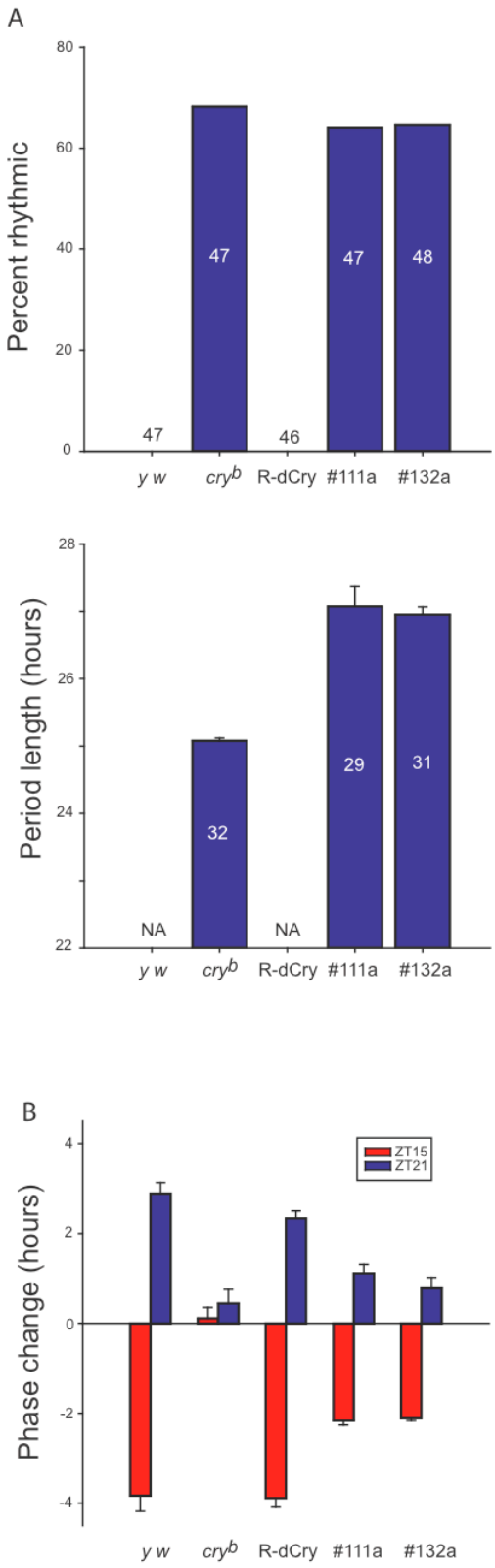


Supplementary Figure S1. Sequence alignment of *Drosophila* Cry (dCry), monarch Cry1 (dpCry1) and monarch Cry2 (dpCry2). Peptide sequences of dCry (AAC83828), dpCry1 (AAX58599), and dpCry2 (ABA62409) were aligned using Pileup in the Genetics Computing Group software package. Residues identical in all three proteins were colored red, and residues similar in two out of three proteins were colored blue. The three tryptophans that comprise the trp triad are marked by asterisks.



Supplementary Figure S2. *tim-GAL4* driven transgenic expression of *Drosophila* UAS-dCryW342F mutations partially restores circadian behaviour in *cry*^{b} flies.
We assayed the effects of the Drosophila CryW343F mutation on circadian behaviour of flies using two methods.

A, First, we examined the locomotor activity of flies under constant light conditions. Under these conditions, flies with functional Cry have a lengthened circadian period that ultimately leads to arrhytmicity whereas Cry-defective *cry*^{b} flies are behaviourally rhythmic[30].

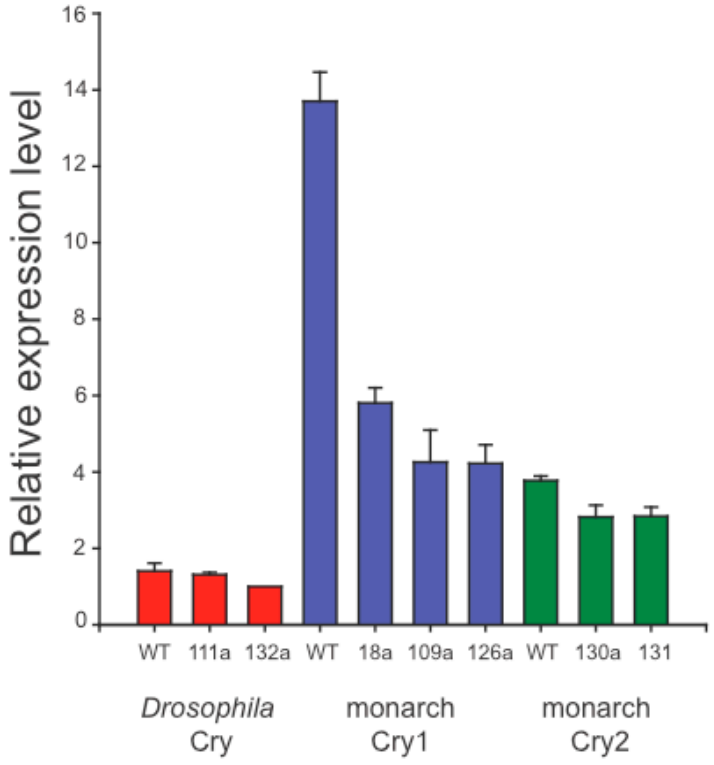
Flies were entrained to a 12-h light:12-h dark cycle for 4 days and then held under constant light conditions (approximately 250 lux) for 6 days. Five days of locomotor activity rhythms were analyzed, beginning 24 hours after the onset of constant light.

Wild-type *y w* flies and dCry-rescued *cry*^{b} flies (R-dCry) were arrhythmic, while most of the *cry*^{b} flies and the two *UAS-dcryW342F* lines (#111a and #132a) expressed circadian rhythms (upper panel). However, the #111a and #132a lines had a period length that was an approximately 2 hours longer than that in *cry*^{b} controls (lower panel). Numbers in each bar indicate the number of flies tested. The period length for *y w* and R-dCry was labelled 'NA' in lower panel because they were all arrhythmic.

B, Second, we examined the ability of light pulses under constant dark conditions to phase-shift the circadian clock of flies expressing the *Drosophila* CryW432F transgene.

16 males per genotype per group were entrained to a 12-h light:12-h dark cycle in 250-500 lux for four full days before receiving a 1-h, 1000 to 1200 lux light pulse at Zeitgeber time (ZT)15 or ZT 21. A "no-pulse" control group remained in the dark. Flies were then held under constant dark conditions for 6 days. Data were collected using the TriKinetics Drosophila Activity Monitor (DAM) system and analyzed. Phase shifts were determined for each genotype by taking the average delay or advance of the three peaks of activity after the light pulse and subtracting the average delay or advance of the three peaks before the light pulse. The first peak of activity directly after the light pulse was not included in the average.

Lines #111a and #132a partially rescued phase advances and delays after a 1-h light pulse at Zeitgeber time (ZT) 15 (red bars) or ZT 21 (blue bars), respectively. Near complete rescue was attained by R-dCry, compared to wild-type *y w* flies. The *cry*^{b} flies did not exhibit any phase changes. Phase changes: positive numbers are advances, negative numbers are delays. Each value is the mean ± s.e.m of three independent experiments.



Supplementary Figure S3. Protein levels for the expressed *UAS* transgenes under control of the *tim-GAL4* driver in *cry*^{b} flies.

Flies were collected at night at Zeitgeber time (ZT) 21, with light on from ZT 0/24 to ZT 12. Head protein extracts were analyzed by western blot, quantified by chemiluminescence, and normalized against α-tubulin. Protein samples from the *Drosophila* Cry expressing line and the two independent dCryW342F lines (111a and 132a) were probed with *Drosophila* CRY-specific antibody RB14[31]. Protein samples from the monarch Cry1 expressing line and the three independent dpCry1W328F lines (18a, 109a, and 126a) were probed with monarch CRY1-specific antibody GP37[32]. Protein samples from the monarch Cry2 expressing line and the two independent dpCry2W345F lines (130a and 131) were probed with monarch CRY2-specific antibody GP51[16]. The same protein samples from the *Drosophila* Cry, monarch Cry1 and monarch Cry2 expressing lines from the previous three blots were also probed with an anti-MYC antibody, as all three expressed wild-type proteins are MYC tagged. All protein levels could then be standardized to the anti-MYC blot and the lowest value was normalized to 1. Each value is the mean ± s.e.m of three independent experiments.

Supplementary References

[30] Emery, P. *et al.* Drosophila CRY is a deep brain circadian photoreceptor. *Neuron* **26**, 493-504 (2000).

[31] Rush, B. L., Murad, A., Emery, P. & Giebultowicz, J. M. Ectopic CRYPTOCHROME renders TIM light sensitive in the Drosophila ovary. *Journal of Biological Rhythms* **21**, 272-278 (2006).

[32] Sauman, I. *et al.* Connecting the navigational clock to sun compass input in monarch butterfly brain. *Neuron* **46**, 457-467 (2005).